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Spatial distribution of *Aspergillus flavus* and its toxigenic strains on commercial cottonseed from south Texas and its relationship to aflatoxin contamination

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The structure of *Aspergillus flavus* communities associated with south Texas cottonseed was determined by analysing samples from 178 truckloads of commercial cottonseed from 35 gins, extending from Fort Bend County in the north to the Rio Grande Valley in the south, from September 1999 to October 2001. The number of colony-forming units (CFU) of *A. flavus* on the cottonseed, and the percentage of S strain (%S) were both correlated with aflatoxin contamination of cottonseed. The number of CFU differed between both regions and seasons, while %S differed only between regions. Comparison of maps of CFU and %S revealed that CFU shows a higher variation across years, while %S shows higher spatial variation. The Rio Grande Valley had significantly lower CFU and %S strain than the Coastal Bend and Upper Coast regions. Cottonseed produced in 1999 had significantly more *A. flavus* than that produced in either 2000 or 2001. Identification of factors dictating geographical variation in S-strain incidence may provide insights that will lead to improved aflatoxin management.

Keywords: aflatoxin, cotton, geographic information systems, kriging, spatial analysis

Introduction

Regulatory limitations on the quantity of aflatoxins permitted in food and feed exist throughout most of the world (Park *et al.*, 1988; van Egmond, 2002). Where contamination is common, diverse communities of aflatoxin-producing fungi reside. These fungi are asexual and belong to *Aspergillus* section *Flavi* (Cotty *et al.*, 1994). Communities of section *Flavi* differ by region in both species composition and aflatoxin-producing potential. *Aspergillus flavus*, the most common aflatoxin-producing species, can be divided into two strains based on morphological, genetic and physiological criteria (Cotty, 1989; Bayman & Cotty, 1993; Egel *et al.*, 1994; Ehrlich *et al.*, 2003). The S strain produces numerous small sclerotia (average diameter <400 μm) and high levels of aflatoxins, while the L strain produces fewer, larger sclerotia and, on average, less aflatoxin (Cotty, 1989; Cotty, 1997; Garber & Cotty, 1997). The *A. flavus* S strain is suspected to be an important causal agent of aflatoxin contamination in several areas worldwide, including Arizona (Cotty, 1989; Cotty, 1997; Orum *et al.*, 1999), Texas (Horn & Dorner, 1998; Cotty *et al.*, 2001), Louisiana, Mississippi and Alabama (Cotty, 1997) in the USA; Thailand in Southeast Asia (Saito *et al.*, 1986); Benin in Africa (Cardwell & Cotty, 2002); and Argentina in South America (Novas & Cabral, 2002).

Aflatoxin contamination of cottonseed costs millions of dollars annually. It has long been a concern for the USA cottonseed industry because a small proportion of aflatoxin in feed is transferred to the milk of dairy cows in the slightly modified form aflatoxin M1 (Allcroft & Carnaghan, 1963; van Egmond, 1989). Cottonseed is a preferred feed for dairy cows, and US regulations prohibit aflatoxin concentrations over 0·5 ng g^{-1} in milk (Park & Troxell, 2002). Milk exceeding that limit may be dumped and the producing dairy placed under quarantine. As dairies pay a premium for cottonseed with aflatoxin levels below the limits, aflatoxin content is the most important factor determining seed value in areas where aflatoxin contamination of cottonseed is common (Cotty, 2001). In the USA, aflatoxin contamination of cottonseed is most severe in the desert production regions of Arizona and southern California, and in south Texas (Schroeder & Boller, 1973; Russell, 1982; Cotty, 2001).

Geographic information systems (GIS) and geostatistics describe, analyse and display variables spatially. One way these valuable tools help in the solution of real-world problems and improve management of resources is by revealing causal relationships among spatially variable

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factors. Geostatistics describe spatial continuity and adapt linear regression techniques to estimate values at unsampled locations (Isaaks & Srivastava, 1989; Cressie, 1993). Kriging is an interpolation technique for spatially correlated data (Myers, 1991). Geostatistics has several applications in plant pathology, including descriptions of epidemics (Jaime-Garcia *et al.*, 2001; Wu *et al.*, 2001); regional risk assessment for virus management (Nelson *et al.*, 1994); descriptions of pathogen populations (Orum *et al.*, 1999; Jaime-Garcia *et al.*, 2001); and aflatoxin contamination across landscapes (Jaime-Garcia & Cotty, 2003). GIS describes objects in terms of: (i) real-world position with respect to a known coordinate system; (ii) attributes unrelated to position (such as temperature or pathogen density); and (iii) spatial relationship (topological relations) (Fedra, 1993; Seem, 1993).

Aflatoxin contamination of commercial cottonseed in south Texas presents both temporal and spatial variation (Jaime-Garcia & Cotty, 2003). Temporal variation occurs both between and within seasons. The community structure of *A. flavus* is also known to present both temporal and spatial variation (Orum *et al.*, 1999; Bock *et al.*, 2004), but relationships of aflatoxin contamination to fungal community structure have not been described. Furthermore, communities of aflatoxin-producing fungi associated with cotton crops in south Texas have not been characterized. The current study sought to relate incidences and spatial distributions of *A. flavus* strain S on cottonseed in south Texas to aflatoxin contamination.

Materials and methods

Sampling and culturing

Cottonseed samples from trucks transporting cottonseed (18–35 tonnes per truck, 23 tonnes average) from gins in south Texas were taken upon receipt at the Valley Co-op Oil Mill, Harlingen, TX, USA during the seasons of 1999–2001. Samples were taken on a weekly basis, with 30–92 samples taken each season from the Coastal Bend, Upper Coast and Rio Grande regions, except in 1999 when the Rio Grande region was not sampled (Table 1). Six cores, each consisting of 3–5 kg seed, were taken from different locations in each truck (18–30 kg total), mixed, and immediately split and subdivided, resulting in a 1–1·5-kg sample for laboratory analysis in New Orleans, LA, USA. Samples were stored dry initially for 1–3 weeks at room temperature (24°C). Approximately 20 g cottonseed was washed in 500 mL water with 0·006% Triton X-100 by shaking at 250 r.p.m. for 30 min. Members of *Aspergillus* section *Flavi* were isolated from the washings using a dilution-plate technique on a modified Rose Bengal agar (Cotty, 1989). This procedure was repeated three to six times, depending on the number of isolates obtained from each sample. Isolates were assigned to *A. flavus* S or L strain, based on colony characteristics and strain morphology (Cotty, 1989) after subculturing on 5% V8 juice agar (pH 5·2) for 5–7 days at 31°C. Quantities of *A. flavus* on cottonseed were enumerated as colony-forming units (CFU) of *A. flavus* per g cottonseed. The percentage of isolates belonging to strain S of *A. flavus* (%S) was obtained by dividing the number of strain S by the total number (15 isolates) of *A. flavus* obtained for each sample and multiplying by 100. The average of the multiple samples per gin taken during the season was used for all analyses. The average aflatoxin content in ng g^{-1} for all truckloads from each gin and season was obtained as reported previously (Jaime-Garcia & Cotty, 2003) from the Valley Co-op Oil Mill. Trucks were sampled as above. After subdividing, seed (*c.* 10 kg) was immediately decorticated and the kernels were transferred to an on-site laboratory, where the total aflatoxin content was determined within an hour of arrival by a Federal Grain Inspection Service (FGIS)-approved method. The two methods used during the course of the study were Aflatest (Vicam) or Veratox (Neogen Corporation).

Data analysis

The study area was over 450 km long by 100 km wide, extending from the Rio Grande Valley in the south to Fort Bend County in the north. The total area was divided into three geographical regions (Rio Grande Valley, Coastal Bend and Upper Coast). ANOVA was used to assess differences among areas and years for CFU, the natural logarithm of CFU, and %S of *A. flavus*. Means were separated statistically with Tukey's honestly significant difference test. Pearson's correlation analyses for the variables %S, CFU, natural logarithm of CFU, aflatoxin content and natural logarithm of aflatoxin content were performed using SAS ver. 8·0 (SAS Institute Inc.). Multiple linear regression analyses were performed using the stepwise procedure (SAS ver. 8·0). Annual average aflatoxin content of cottonseed from the gins sampled was used for

Table 1 Number of gins and cottonseed truckloads sampled, and number of *Aspergillus flavus* isolates analysed, during the 1999, 2000 and 2001 seasons in south Texas

	1999			2000			2001		
Area	Gins	Trucks	Isolates	Gins	Trucks	Isolates	Gins	Trucks	Isolates
Rio Grande	–	–	–	13	16	252	10	20	583
Coastal Bend	6	18	180	12	25	360	9	42	281
Upper Coast	4	12	120	6	15	268	7	30	430
South Texas	10	30	300	31	56	880	26	92	1249

both Pearson's correlation and multiple linear regression analyses with *A. flavus* community structure (%S; CFU g^{-1}; natural logarithm of CFU g^{-1}) as independent variables. Few data points had S-strain incidences >40%, and increases in aflatoxin content associated with increases in %S reached a plateau at this level. Therefore Pearson's correlation and multiple linear regression analyses were performed with the entire data set, as well as with samples having a %S value >40% excluded.

Spatial analyses

To determine if geographical location significantly influences strain composition and the magnitude of *A. flavus* communities, spatial analyses of *A. flavus* on cottonseed surfaces from different areas of south Texas were performed using geostatistics (Nelson *et al.*, 1999). These analyses were performed on the annual average data of each gin for %S and CFU of *A. flavus*. Sample support size influences geostatistical analyses: although geostatistical calculations use point data, these data may be derived from either single analyses representing a square metre, or many analyses on locations covering many kilometres. In geostatistics, support is the actual physical dimensions represented by each data point. Increasing support size with multiple measurements decreases erratic variability that may result from scales not under investigation. (Isaaks & Srivastava, 1989; Myers, 1991). Considering this, it is assumed that most of the cottonseed from a given gin was obtained from a vicinity of *c.* 25 km, so each set of gin data is supported by several samples (truckloads) that probably came from fields within this range. Each gin analysed was georeferenced in the Universal Transverse Mercator projected coordinate system.

Variogram analyses

Model variograms describe spatial continuity using the parameters 'range', 'nugget' and 'sill' to fit experimental variogram values derived from the data (Isaaks & Srivastava, 1989; Journel, 1989). Range describes the distance over which the spatial autocorrelation occurs; nugget describes variability at short ranges and the experimental error; and sill describes the spatial variability of the random variable over the range. Omnidirectional variogram models were obtained using the VARIOWIN 2·1 software (Y Pannatier, Institute of Mineralogy and Petrography, University of Lausanne, Switzerland) for CFU and %S. Mean sample values for each gin during each season were used in these analyses. Variogram models describe the spatial autocorrelation and provide function and parameters for surface interpolation with kriging. Variogram equations, and procedures to develop experimental variograms and fit model variograms, followed procedures described previously (Isaaks & Srivastava, 1989; Journel, 1989; Myers, 1991; Deutsch & Journel, 1992; Cressie, 1993). The maximum distance between sample locations (gins) was *c.* 450 km, and the minimum distance was *c.* 2·5 km.

Kriging and map display

Variogram models for %S and CFU for each season were used in ordinary block kriging to obtain surface maps of %S and CFU. Block kriging uses point data and a variogram model to estimate values in blocks or cells. ARCGIS ver. 8·0 (ESRI) was used for block kriging. Interpolation of values for unsampled areas was performed on a 2 × 2-km cell grid with a search neighbourhood of 60 000 m and a maximum of 12 sample locations. Season averages of %S and CFU for each gin were used. ARCGIS ver. 8·0 was used to create views and layouts of the kriged values with previously digitized features as background.

Results

Communities of *A. flavus* resident on cottonseed surfaces from gins in south Texas varied according to region and year (Tables 2 and 3; Fig. 1). Both quantity (CFU) and strain composition (%S) of *A. flavus* influenced aflatoxin contamination of cottonseed (Tables 4 and 5).

Magnitude of *A. flavus* communities

The magnitude of *A. flavus* (CFU) on cottonseed in south Texas differed significantly both among seasons and across regions (Tables 2 and 3). The 1999 season had significantly higher CFU than the 2000 and 2001 seasons (Table 2). Similar CFU results were obtained for each region when analysed separately. Both Coastal Bend and Upper Coast regions had significantly higher CFU in the 1999 season than in the 2000 and 2001 seasons. Both the Upper Coast and the Coastal Bend regions had significantly higher CFU than the Rio Grande Valley when data from all seasons were analysed together. There were no significant differences in CFU between regions when analysed by individual season (Tables 2 and 3).

Incidence of strain S

Average values of S-strain incidence (%S) differed significantly among regions. Similar differences occurred for each individual year (Tables 2 and 3). The %S for the Rio Grande Valley was consistently lower than for the Coastal Bend and Upper Coast regions (Table 2). For the 1999 season, when data were not obtained from the Rio Grande Valley, the Coastal Bend region had significantly higher %S than the Upper Coast (Table 2). In general, %S between years for a given region had little variation (<5%, Tables 2 and 3). Significant differences were detected only for the Coastal Bend region between 1999 and both 2000 and 2001 (Table 2).

Relationships between aflatoxin contamination and *A. flavus* community parameters

Pearson's correlation analysis indicated significant positive correlations of aflatoxin content with both %S and CFU (Table 4). The correlation coefficients for the average

Table 2 Colony-forming units (CFU) of *Aspergillus flavus* and percentage *A. flavus* strain S in commercial cottonseed from gins in south Texas from 1999 to 2001

Regions	1999	2000	2001	1999–2001
A. flavus CFU g^{-1}				
South Texas[a]	3077 A[b]	570 B	771 B	1204
South Texas	3077 A	522 C	733 B	1063
Rio Grande Valley		404 A (a)	598 A (a)	512 (b)
Coastal Bend	3369 A (a)	602 B (a)	694 B (a)	1240 (a)
Upper Coast	2638 A (a)	515 B (a)	877 B (a)	1153 (a)
Percentage strain S				
South Texas[a]	38·0 A	27·6 A	30·3 A	31·2
South Texas	38·0 A	22·9 B	26·2 B	27·2
Rio Grande Valley		10·4 A (b)	10·0 A (b)	10·2 (b)
Coastal Bend	46·1 A (a)	27·9 B (a)	32·2 B (a)	33·9 (a)
Upper Coast	25·8 A (b)	27·1 A (a)	27·6 A (a)	27·1 (a)

[a]Not including data from the Rio Grande Valley.

[b]Averages (based on n = gin values in Table 1) with the same letter are not significantly different by Tukey's HSD test ($\alpha = 0{\cdot}05$). Capital letters indicate differences among years (columns) for each region and for south Texas. Lower-case letters in parentheses indicate differences among regions (rows) for each year and for the period under study.

Table 3 Linear models (ANOVA) for colony-forming units of *Aspergillus flavus* and percentage of *A. flavus* strain S in commercial cottonseed from gins in three regions of south Texas from 1999 to 2001

Source	Model df	Error df	F	$P > F$
Percentage strain S				
Regions (1999)	1	28	6·52	0·0164
Regions (2000)	2	52	8·12	0·0009
Regions (2001)	2	87	10·25	0·0001
Regions (1999–2001)	2	172	21·45	<0·0001
Years (Rio Grande Valley)	1	31	0·01	0·9253
Years (Coastal Bend)	2	82	5·09	0·0082
Years (Upper Coast)	2	54	0·05	0·9510
Years (south Texas)	2	172	6·36	0·0022
Years (south Texas)[a]	2	139	2·85	0·0612
A. flavus CFU g^{-1}				
Regions (1999)	1	28	1·25	0·2738
Regions (2000)	2	53	2·86	0·0664
Regions (2001)	2	88	2·51	0·0872
Regions (199–2001)	2	174	9·55	0·0001
Years (Rio Grande Valley)	1	34	2·98	0·0934
Years (Coastal Bend)	2	81	19·02	<0·0001
Years (Upper Coast)	2	54	5·95	0·0046
Years (south Texas)	2	174	29·79	<0·0001
Years (south Texas)[a]	2	138	24·43	<0·0001

[a]Rio Grande Valley data not available.

of the 3 years of study were $r = 0{\cdot}43$ and $r = 0{\cdot}54$ for %S and CFU, respectively. The correlation coefficient for aflatoxin content with CFU was improved to $r = 0{\cdot}79$ when natural logarithms for both aflatoxin content and CFU were used (Table 4). Correlation coefficients differed according to season. In the highly contaminated season of 1999, when CFU were constantly high, %S had its highest correlation coefficient ($r = 0{\cdot}65$) and CFU its lowest ($r = 0{\cdot}33$). The %S had its lowest correlation coefficient in the 2001 season (Table 4). Correlation coefficients for %S were increased by excluding samples containing >40% of the S strain from the analysis (Table 4).

Multiple linear regression analyses by the stepwise method of SAS indicate that the magnitude (CFU) and composition (%S) of the *A. flavus* population infecting cottonseed are major factors related to contamination of cottonseed by aflatoxins (Table 5). Both CFU and %S are included in the regression model for aflatoxin contamination. In general, the transformation of aflatoxin contamination and CFU data to their natural logarithms for the linear regression analyses greatly improved the regression models (Table 5). The best model describing the effects of *A. flavus* populations on aflatoxin contamination in south Texas cottonseed from all seasons studied was obtained when the natural logarithm of aflatoxin contamination was analysed against the natural logarithm of CFU (ln CFU) and the %S ($R^2 = 0{\cdot}66$; $P < 0{\cdot}0001$, Table 5). As environmental factors vary with season, multiple regression analyses were also performed for individual seasons. Models varied by season. In the 1999 season, when CFU were high and less variable than in other years, %S was the only factor significantly explaining aflatoxin variation ($R^2 = 0{\cdot}43$). In 2000, both %S and ln CFU were included in the model ($R^2 = 0{\cdot}58$), while in 2001 ln CFU was the only significant factor ($R^2 = 0{\cdot}54$, Table 5). Linear regression models were greatly improved in the 1999 and 2000 seasons, when the data set analysed was limited to include only data with %S up to 40%, giving $R^2 = 0{\cdot}84$ and 0·67, respectively (Table 5). Likewise, in the 2001 season the model included the variable %S when it was limited to include only samples with a value up to 40%. All the variables included in the models were individually significant at $P \leq 0{\cdot}05$.

Spatial patterns of %S and CFU in south Texas

Geostatistical analyses indicated spatial continuity for both %S and CFU, with intraseason variation in their

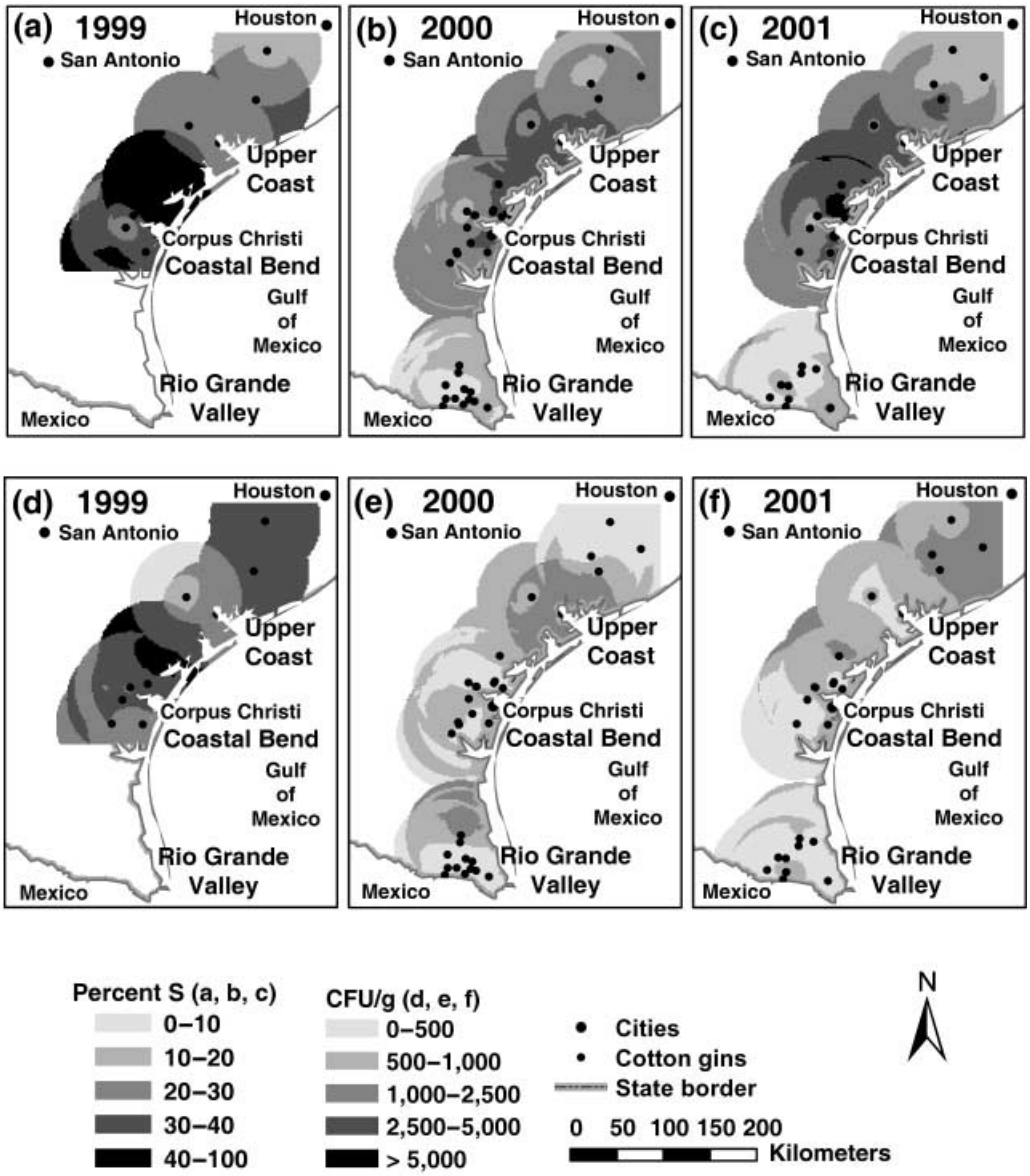

Figure 1 Spatial patterns of (a–c) percentage of *Aspergillus flavus* strain S (%S); (d–f) number of colony-forming units (CFU) of *A. flavus* per g cottonseed in south Texas during the seasons of (a,d) 1999; (b,e) 2000; and (c,f) 2001. Data from the 1999 season are based on few sample data points and should be interpreted with caution.

range of influence. Modelled parameters of variograms for %S and CFU are presented in Table 6. All the variograms for both %S and CFU for the 3 years of study were of the spherical type, with goodness of fit ranging from 0·005 to 0·025 (based on weighted mean-square standardized, values close to zero indicate good fit). Variogram ranges for both %S and CFU were from 27 000 to 55 000 m. Variograms from 2001 had shorter ranges for both %S (27 000 m) and CFU (38 300 m) than those from 1999 and 2000 (Table 6). The highest spatial variation occurred in 1999 for both %S and CFU, with a total sill (nugget + sill) of 485 and 3 600 000, respectively. The lowest spatial variation occurred in 2000 for %S (total sill 120) and in 2001 for CFU (total sill 204 000) (Table 6).

When the data for each season are displayed on maps generated by ordinary block kriging, recurrent patterns are apparent for %S, but not for CFU (Fig. 1). The area extending from the south-eastern part of the Coastal Bend to the southern part of the Upper Coast shows a %S >30%, while the southern part of the Rio Grande Valley shows a %S <20% in the 3 years of study. Although CFU did not show recurrent spatial patterns each year, it did display spatial continuity with distinct high-incidence areas (Fig. 1). Among the years studied, *A. flavus* propagule densities were greatest in 1999. Most of the study area

Table 4 Pearson's correlation coefficients and probability of significance for relationships of cottonseed aflatoxin content with the quantity (colony-forming units, CFU) and composition (percentage *Aspergillus flavus* composed of the S strain, %S) of *A. flavus* from cottonseed from south Texas

	Aflatoxin				ln Aflatoxin[c]			
Variable	1999[a]	2000	2001	1999–2001	1999	2000	2001	1999–2001
%S	0·65[b]	0·43	0·08	0·43	0·66	0·40	0·002	0·40
	0·04	0·01	0·69	<0·001	0·04	0·02	0·99	<0·001
CFU	0·33	0·57	0·50	0·54	0·29	0·57	0·43	0·54
	0·34	<0·001	0·009	<0·001	0·40	<0·001	0·03	<0·001
ln CFU	0·23	0·72	0·68	0·66	0·18	0·72	0·73	0·79
	0·53	<0·001	<0·001	<0·001	0·61	<0·001	<0·001	<0·001
When %S is limited up to 40%								
%S	0·92	0·58	0·56	0·48	0·94	0·58	0·51	0·54
	0·004	*0·001*	*0·009*	*<0·001*	*0·002*	*0·001*	*0·02*	*<0·001*

[a]Number of observations (*n*) for each year varies based on the gin value in Table 1.
[b]Normal text, correlation coefficient; italic text, probability of significance.
[c]Natural logarithm.

Table 5 Multiple linear regression models, coefficient of determination (R^2) and probability of significance ($P > F$) for aflatoxin content (Y) and natural logarithm of aflatoxin content (ln Y) in cottonseed from south Texas

Year	Regression model[a]	R^2	$P > F$
1999–2001	ln Y = 0·01 + (0·012 × %S) + (0·48 × ln CFU)	0·66	<0·0001
1999	Y = 70·3 + (1·44 × %S)	0·43	0·0407
2000	Y = –17·8 + (0·19 × %S) + (5·81 × ln CFU)	0·58	<0·0001
2001	ln Y = 1·16 + (0·34 × ln CFU)	0·54	<0·0001
When %S is limited up to 40%			
1999	Y = 19·8 + (3·52 × %S)	0·84	0·0036
2000	Y = –18·8 + (0·37 × %S) + (5·5 × ln CFU)	0·67	<0·0001
2001	Y = –29·0 + (0·47 × %S) + (8·2 × ln CFU)	0·53	0·0008

[a]%S = Percentage of *A. flavus* isolates belonging to the S strain; CFU = colony-forming units of *A. flavus*; ln CFU = natural logarithm of CFU.
Number of observations (*n*) for each year varies based on the gin value in Table 1.

had average propagule densities >2500 CFU g^{-1} in 1999. In both 2000 and 2001, propagule densities throughout most of the study area were less than 1000 CFU g^{-1} (Fig. 1).

Discussion

Both *A. flavus* population size and structure have major roles in aflatoxin contamination of cottonseed in south Texas. Results from the current study suggest that seasonal variation of aflatoxin contamination is driven by factors that influence *A. flavus* growth and reproduction, as quantified by the density of *A. flavus* propagules on crop surfaces. The data also support variation in population structure, as reflected in the percentage of *A. flavus* communities composed of the S strain, as a factor closely related to spatial variation in aflatoxin contamination. The quantity of *A. flavus* propagules on cottonseed varied between seasons and regions (Tables 2 and 3), but quantitative differences influenced aflatoxin contamination across seasons, although not among regions. In 1999, a season with high aflatoxin contamination (Jaime-Garcia & Cotty, 2003), there were significantly higher CFU than in the 2000 and 2001 seasons (Table 2; Fig. 1). Although the average CFU for the three seasons shows a significant difference in CFU between regions (Tables 2 and 3), this may not be real as there were no data for the Rio Grande Valley in the high-CFU-incidence season of 1999. When data from 1999 were excluded from the analysis, only the Upper Coast region was significantly different from the Rio Grande Valley (Table 2), and there were no significant differences in CFU between regions when data were analysed by individual season.

The most likely cause of variation in *A. flavus* CFU is precipitation during the time when cotton is mature, either in the field or after harvest. The 1999 cotton-cropping season (June–August) had high precipitation (360 mm) compared with 2000 (86 mm) and 2001 (140 mm) (Crop Weather Program for south Texas, Texas A&M University, Agriculture Research and Extension Center, Corpus Christi, TX, USA). Higher precipitation at the mature crop stage might increase the population of *A. flavus* (Bock *et al.*, 2004) and thus the severity of the second phase of contamination (Bock & Cotty, 1999; Cotty, 2001). Precipitation on the mature crop has already been shown to covary with contamination in south Texas (Jaime-Garcia & Cotty, 2003).

Table 6 Variogram models of percentage of *Aspergillus flavus* isolates belonging to the S strain (%S) and colony-forming units (CFU) of *A. flavus* on cottonseed from south Texas, 1999–2001

Variable/ year	Lag distance		Type	Nugget	Sill	Range (m)	IGF[a]
	Interval	Maximum					
%S							
1999[b]	11 500	92 000	Spherical	60	425	50 000	
2000	10 500	84 000	Spherical	50	70	55 000	0·025
2001	8800	70 400	Spherical	0	240	27 000	0·005
Colony-forming units (CFU)							
1999[b]	10 200	81 600	Spherical	1 000 000	2 600 000	50 000	
2000	10 350	82 800	Spherical	45 000	264 000	55 000	0·01
2001	10 100	80 800	Spherical	10 000	194 000	38 300	0·018

[a]IGF = Indicative goodness of fit based on a weighted mean square standardized, where values close to zero indicate good fit. The 1999 season does not present an IGF because it had fewer sample points than required for a reliable variogram.
[b]The 1999 season had only 11 sampled points, thus the variogram is considered not very reliable and caution should be exercised; these variograms are included for purposes of interpolation and map display of %S and CFU data.

Clear geographical structure in the distribution of the S strain on commercial cottonseed in south Texas is revealed by the geostatistical analyses reported here. The S strain is markedly less common on crops from the lower Rio Grande Valley than on Coastal Bend Area crops. Temporal variation in %S across the 3 years of study was not detected (Table 2), indicating that the population structure of *A. flavus* is relatively stable with time, at least over the few years of this study. Evidence for long-term change in %S has not been reported, but that possibility has not been discarded. The higher %S obtained for the Coastal Bend area for the 1999 season, compared with the other seasons and the Upper Coast area (Table 2; Fig. 1), might be because, for that area and season, fewer gins were sampled – and they were mainly those gins that had the highest %S during the other seasons. Data from this study indicate geographical substructuring (Fry *et al.*, 1992; Jaime-Garcia *et al.*, 2001) of *A. flavus* communities, with patches containing increased incidence of the S strain (Table 2; Fig. 1). Patchy distribution of the S strain of *A. flavus* has been reported on several scales, including among different US states (Cotty, 1997; Horn & Dorner, 1998, 1999); among agricultural areas in one state (Bigelow *et al.*, 2001); and within an agricultural area (Orum *et al.*, 1999). It is not clear what factors dictate S-strain distribution. Although increased elevation has been associated with reduced S-strain incidence within agricultural areas in Arizona (Bigelow *et al.*, 2001), this is not a factor in south Texas. For instance, areas with similar altitude (e.g. Rio Grande Valley, the Coastal Bend and Upper Coast) differed in incidence of %S from very low to very high incidences (Fig. 1), suggesting that crop-rotation practices and soil type might be other factors affecting S-strain distribution.

Correlation analysis and multiple linear regression models confirmed the association of both %S and CFU with contamination. In 1999, aflatoxin was highly correlated with %S, but not with CFU (Table 4). As a result, only %S met the criteria for inclusion in the stepwise multiple linear regression model (Table 5). Failure of CFU to correlate with contamination may be due to the uniformly high CFU in all the samples from 1999. In 2000 and 2001, CFU varied considerably among samples, and both CFU and %S met the criteria for inclusion in the multiple linear regression models (Table 5). It has been shown previously that incidence of rain on the mature crop is a primary factor dictating variation across years in cottonseed aflatoxin content in south Texas (Jaime-Garcia & Cotty, 2003). The results of the current study suggest that these effects across years result from influences on fungal growth and reproduction, as measured by CFU. Schroeder & Boller (1973) previously suggested that environmental factors affect contamination primarily through influences on growth and reproduction of the causal fungi.

There is some variation in contamination that is not explained by CFU. Incidence of the S strain explains a component of this variation, as illustrated by the correlation ($r = 0{\cdot}92$) between contamination and %S during 1999, when CFU was uniformly high and was not correlated with contamination ($r = 0{\cdot}33$). The S strain of *A. flavus* is the most important contributor to the average aflatoxin-producing potential of fungal communities resident in agricultural areas of Arizona and elsewhere (Cotty, 1997), and the current data suggest that the S strain may be of similar importance in parts of south Texas, and where it appears to be the main factor associated with spatial variation of aflatoxin contamination. For instance, the northern portion of the Coastal Bend and the southern portion of the Upper Coast, in general, had the highest %S in cottonseed during the 3 years of the study, and these areas had the highest aflatoxin contamination (Jaime-Garcia & Cotty, 2003).

The importance of the S strain of *A. flavus* as an aflatoxin producer in Arizona is well known (Cotty, 1989, 1994, 1996, 1997; Garber & Cotty, 1997), and aflatoxin contamination resulting from S-strain infection has been documented in glasshouse and laboratory studies (Cotty, 1996, 1997; Garber & Cotty, 1997). However, relationships

between incidences of S-strain propagules on commercial crops and contamination have not been shown previously. In this study, correlation analyses indicate that the *A. flavus* S strain is a significant factor influencing aflatoxin contamination in south Texas. When both correlation analyses and multiple linear regression analyses were limited to include only samples with up to 40% S strain, the relationship between aflatoxin contamination and %S was greatly improved (Tables 4 and 5). Thus crop aflatoxin content increases with %S up to 40% S; but percentages of S strain above 40% occurred too infrequently to assess accurately if percentages >40% increased contamination. Research is needed to address the relationship of strain S to aflatoxin contamination of crops in south Texas. This is especially important when developing management procedures if the life cycles of the potential causal agents vary. Such variation has been demonstrated between the L and S strains of *A. flavus* in Arizona (Orum *et al.*, 1999; Bock *et al.*, 2004).

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